6. TOXICITY IN ANIMALS

This chapter updates the evaluation of animal studies published from 1985 through January 1997. The study by Owen et al. (1987) is not evaluated here because it was reviewed previously in U.S. EPA (1985) as the NTP study (1984), which was subsequently published by Owen et al. (1987).

6.1. SUBCHRONIC TOXICITY

Irons et al. (1986a, b) conducted studies to assess the potential of 1,3-butadiene to induce myelotoxicity by exposing male B6C3F_1 mice and male NIH Swiss mice to 1,250 ppm 1,3-butadiene, 6 h/day, 5 days/week for 6 weeks. Treatment-related hematological changes included decreases in red blood cell counts, total hemoglobin, and hematocrit and increases in mean cell volume and circulating micronuclei in both strains of mice. The observed anemia was not accompanied by significant alterations in mean corpuscular hemoglobin concentration, increases in reticulocyte counts, or increases in the frequency of nucleated erythrocytes in peripheral blood. These hematologic changes were considered to represent a macrocytic-megaloblastic anemia, because they were accompanied by mild megaloblastic changes in bone marrow cells.

Exposure of male B6C3F_1 mice to 1,250 ppm 1,3-butadiene for 6 h/day, 5 days/week, for 6 or 12 weeks did not produce any persistent effects on humoral or cell-mediated immunity (Thurmond et al., 1986). Relative thymus weights were unaffected, but relative spleen weights were decreased 20% and spleen cellularity was decreased 29% in exposed mice. Extramedullary hematopoiesis and erythroid hyperplasia in exposed mice correlated with a twofold increase in thymidine incorporation into spontaneously proliferating splenocytes. Although the number of IgM antibody plaque-forming cells (PFC) per 10^6 splenocytes was unchanged, a 30% decrease in PFC/spleen was observed. Proliferation of alloantigens was similar for 1,3-butadiene-exposed splenocytes and controls. The mitogenic response of mature T lymphocytes to phytohemagglutinin was significantly suppressed after exposure to 1,3-butadiene for 6 or 12 weeks.

6.2. CHRONIC TOXICITY

A 2-year chronic inhalation toxicity and carcinogenicity study on the effects of 1,3-butadiene on B6C3F_1 mice was conducted by NTP (1993). In this study, groups of 70 male and 70 female mice were exposed by inhalation 6 hours/day, 5 days/week to 0, 6.25, 20, 62.5, or 200 ppm 1,3-butadiene for periods up to 103 weeks; groups of 90 male and 90 female mice were similarly exposed to 625 ppm 1,3-butadiene, which was the lowest exposure level in the previous NTP (1984) study. The additional animals in the 625-ppm exposure group were included because high mortality rates, observed previously at this exposure concentration, might interfere with the scheduled interim evaluations. Interim evaluations were conducted at 9 and 15 months.

Mean body weight gains of male and female mice exposed to 6.25-625 ppm 1,3-butadiene for 103 weeks were similar to those of controls. However, concentration-related decreases in survival were seen in male and female mice exposed to concentrations $\geq$20 ppm 1,3-butadiene (Table 6-1, Figures 6-1 and 6-2) primarily due to the development of malignant neoplasms. No female mice exposed to 200 or 625 ppm or male mice exposed to 625 ppm survived to the end of the study. Statistical analysis for the probability of survival was estimated using the Kaplan and Meyer (1958) procedure; the method of Cox (1972) and Tarone's (1975) life table test was used to identify concentration-related trends.

At the 9- and 15-month interim evaluations, no clinical findings other than those associated with lesion development and moribundity were observed. Some statistically significant organ weight changes were observed at interim evaluations in male and female mice exposed to 1,3-

butadiene concentrations ≥62.5 ppm. Effects related to toxicity to reproductive organs are discussed in Chapter 5.

Hematological indices measured at the interim evaluations showed significant ($p \leq 0.05$) decreases in erythrocyte counts, hemoglobin concentration, and packed cell volume in male mice exposed to ≥62.5 ppm and in female mice exposed to ≥200 ppm at 9 months. Mean cell volume was significantly increased in male mice exposed to 625 ppm and in females exposed to ≥200 ppm at 9 months. A similar profile of hematological changes was observed in male and female mice exposed to 625 ppm for 15 months. Increases in the percentage of erythrocytes with Howell-Jolly body inclusions and mean cell hemoglobin were seen at 9 and 15 months. At the 15-month interim evaluation, males exposed to 625 ppm 1,3-butadiene had a significantly increased mean platelet value, a finding that correlated with the development of neoplasms. Because these hematological changes were not associated with increases in reticulocyte counts or in frequency of polychromatic erythrocytes in peripheral blood, they were attributed to a partial or poorly regenerative bone marrow response to decreased levels of circulating erythrocytes. There were no significant changes in total serum enzyme activity of lactate dehydrogenase (LDH) or creatine kinase in mice evaluated at 9 months. LDH values at the 15 month evaluation were increased in males and females exposed to ≥200 ppm. At 625 ppm, LDH-1 and LDH-2 were decreased and LDH-5, the principal enzyme in skeletal muscle and liver, was increased.

Table 6-1. Survival of male and female B6C3F$_1$ mice exposed to 1,3-butadiene by inhalation for 103 weeks

	Concentration (ppm)					
	0	**6.25**	**20**	**62.5**	**200**	**625**
Male						
Animals initially in study	70	70	70	70	70	90
9-Month interim evaluation[a]	10	10	10	10	10	10
15-Month interim evaluation[a]	10	10	10	10	10	7
Natural deaths	6	5	11	12	23	39
Moribund kills	9	6	15	15	23	33
Accidental deaths[a]	0	0	0	0	0	1
Missing[a]	0	0	0	1	0	0
Animals surviving until study termination	35	39	24	22	4[b]	0
Percent survival at end of study[c]	70	78	49	46	8	0
Mean survival(days)[d]	597	611	575	558	502	280
Survival analysis[e]	$p<0.001$	$p=0.430$N	$p=0.044$	$p=0.021$	$p<0.001$	$p<0.001$
Female						
Animals initially in study	70	70	70	70	70	90
9-Month interim evaluation[a]	10	10	10	10	10	8
15-Month interim evaluation[a]	10	10	10	10	10	2
Natural deaths	3	7	11	8	12	33
Moribund kills	10	10	14	31	37	46
Accidental deaths[a]	0	0	1	0	1	1
Animals surviving until study termination	37	33	24	11	0	0
Percent survival at end of study[c]	74	66	50	23	0	0
Mean survival (days)[d]	608	597	573	548	441	320
Survival analysis[e]	$p<0.001$	$p=0.510$	$p=0.013$	$p<0.001$	$p<0.001$	$p<0.001$

[a] Censored from survival analyses.
[b] Includes one animal that died during the last week of the study.
[c] Kaplan-Meier determinations. Survival rates adjusted for interim evaluations, accidental deaths and missing animals.
[d] Mean of all deaths (uncensored, censored, terminal sacrifice).
[e] The result of the life table trend test (Tarone, 1975) is in the control column, and the results of the life pairwise comparisons (Cox, 1972) with the controls are in the dosed columns. A negative trend or lower mortality in a dose group is indicated by N.

Source: NTP, 1993.

Histopathological effects observed at the 9-month evaluations included bone marrow atrophy (depletion of cells) in 50% of males and in 13% of females at the highest concentration (625 ppm). The atrophy increased in severity from mild depletion of hematopoietic cells at 9 months to marked depletion in mice that died or were killed at or before 15 months. An increased incidence of bone marrow hyperplasia and an increased incidence or severity of hematopoiesis of the spleen, liver, and lung occurred in females exposed to the three highest concentrations (≥62.5 ppm). Thymic necrosis (atrophy) and decreased thymus weights were seen at the 9-month evaluation in males and females exposed to 625 ppm. Thymic necrosis also
occurred in females exposed to 62.5 or 200 ppm.

In mice exposed to 1,3-butadiene for 103 weeks, nonneoplastic effects were observed in the bone marrow, liver, testes, ovary, heart, upper respiratory tract, and various other organs.

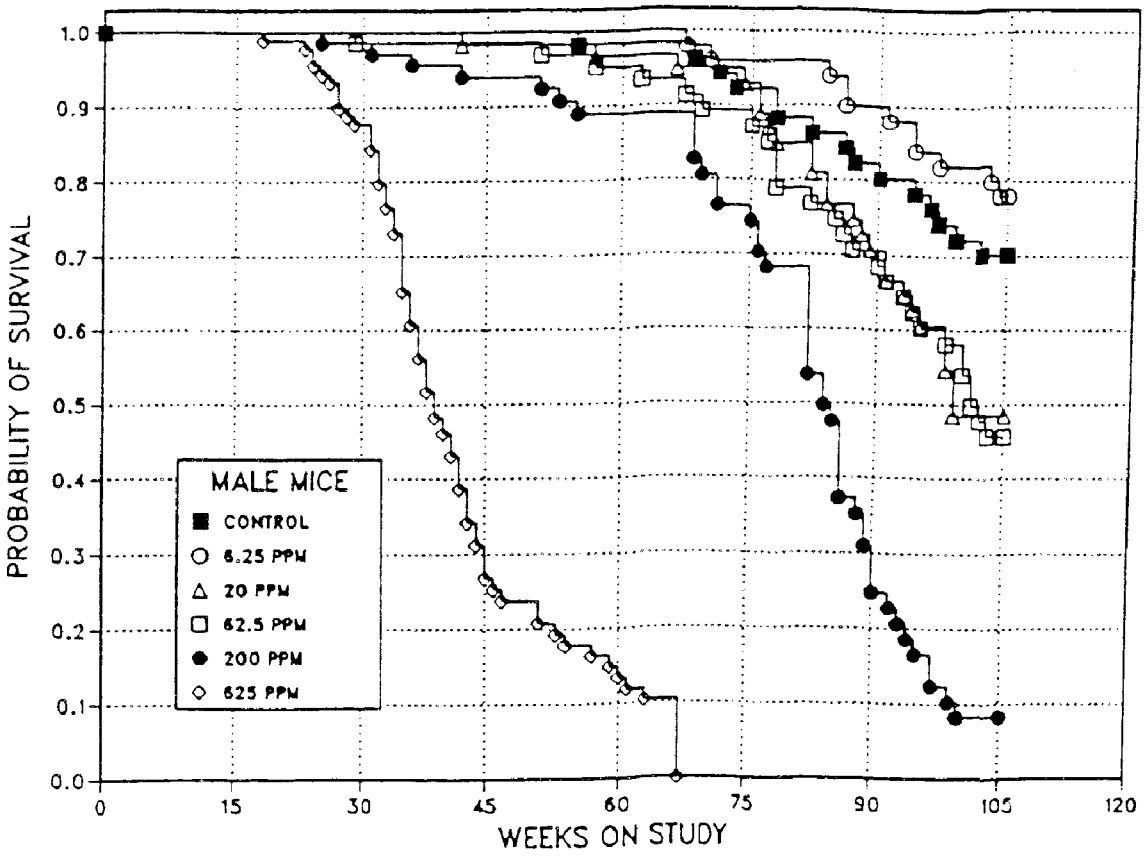

Figure 6-1. Kaplan-Meier survival curves for male B6C3F$_1$ mice exposed to 1,3-butadiene by inhalation for 103 weeks.
Source: NTP, 1993.

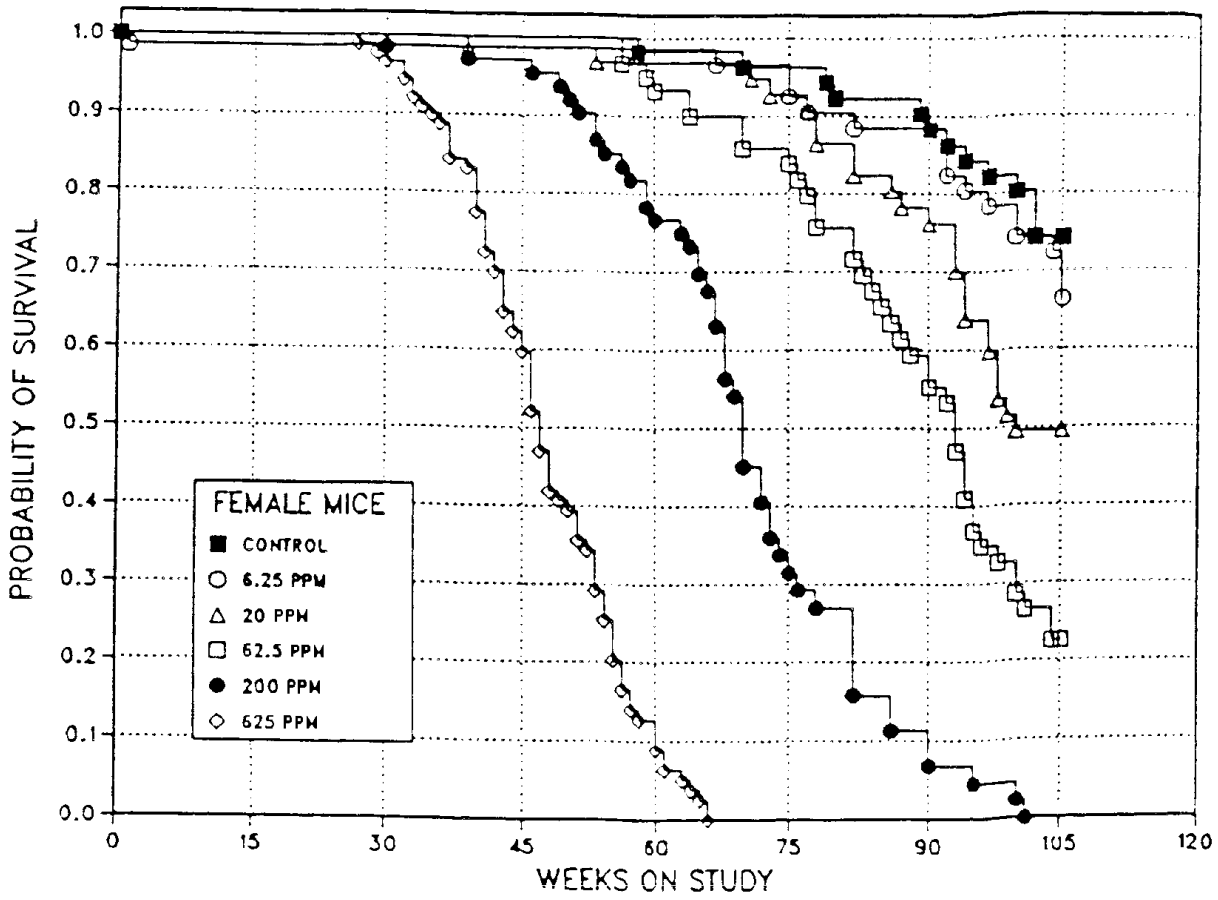

Figure 6-2. Kaplan-Meier survival curves for female B6C3F$_1$ mice exposed to 1,3-butadiene by inhalation for 103 weeks.
Source: NTP, 1993.

Effects in reproductive organs are discussed in Chapter 5. Organ weights, hematological indices, and serum chemistry were not evaluated at 103 weeks.

In the 2-year study, bone marrow atrophy was recorded in 50% of males and 14% of females exposed to 625 ppm.

The incidence of liver necrosis was increased at the higher exposure concentrations in males and females, occurring in 8%, 10%, 16%, 27%, 29%, and 26% of males and in 4%, 4%, 14%, 10%, 38%, and 21% of females exposed to 0 (controls), 6.25, 20, 62.5, 200, and 625 ppm, respectively. Centrilobular hepatocellular necrosis of the liver was seen in 4% and 8% of males exposed to 62.5 and 625 ppm, respectively, and in 2%, 8%, and 9% of females exposed to 62.5, 200, and 625 ppm, respectively. Hepatocellular necrosis was not seen in any of the concurrent controls. Liver necrosis with no particular lobular distribution was found primarily in animals with malignant lymphoma and hemangiosarcoma; centrilobular hepatocellular necrosis was often found in animals described as anemic and in animals with atrial thrombi.

Myocardial mineralization, a lesion of unknown pathogenesis, occurred with increased frequency in both sexes at 625 ppm (males, 27%; females, 14%), but was not observed in controls. A low incidence was observed at the lower concentrations. Myocardial mineralization was also observed in a separate stop-exposure study in which male mice were exposed to 312 ppm 1,3-butadiene for 52 weeks or 625 ppm for 13 or 26 weeks, and observed for periods up to 103 weeks. The incidence of myocardial mineralization for these three exposure groups was 12%, 18%, and 28%, respectively. Details of the stop-exposure study are presented in Section 3.3.

Minimal to mild olfactory epithelial atrophy occurred in females exposed to 625 ppm and in males exposed to concentrations $\geq$20 ppm. However, the incidence in males exposed to 625 ppm was lower than that seen in females. The olfactory epithelial lesions were unilateral at the lower concentrations and bilateral at the higher concentrations. The lesions were similar to those seen in the NTP (1984) study, but osseous or cartilaginous metaplasia was not observed. The investigators considered olfactory nasal atrophy a possibly compound-related lesion.

Compared with controls, mice exposed to 1,3-butadiene exhibited increased incidences of proliferative lesions (hyperplasia) in several organs, including the heart, lungs, forestomach, ovaries, mammary gland, and Harderian gland (Table 6-2). Hyperplasia of the endothelium (cardiac blood vessels), alveolar epithelium, forestomach epithelium (focal), germinal epithelium and granulosa cells of the ovaries, mammary gland, and Harderian gland were all considered preneoplastic lesions. Other preneoplastic lesions identified in the 2-year study were hepatocellular foci (basophilic, clear cell, mixed cell, and eosinophilic) in female mice exposed to 1,3-butadiene. Hepatocellular foci were observed in 16% of controls and in 29%, 38%, 24%, 10%, and 5% of females exposed to 6.25, 20, 62.5, 200, and 625 ppm, respectively. Hyperplastic lesions were also observed in separate studies with male B6C3F_1 mice using variable exposure and durations (stop-exposure experiments). Hyperplasia in these studies occurred primarily in the endothelium (cardiac blood vessels), alveolar epithelium, forestomach epithelium, and Harderian gland (Table 6-3).

Table 6-2. Incidence of hyperplasia in male and female B6C3F$_1$ mice exposed to 1,3-butadiene by inhalation for 103 weeks

Organ/tissue	Sex	Concentration (ppm)					
		0	6.25	20	62.5	200	625
Heart, endothelium	M	0/50 (0%)	1/49 (2%)	0/50 (0%)	2/48 (4%)	4/48 (8%)	5/73 (7%)
	F	0/50 (0%)	2/50 (4%)	1/50 (2%)	4/49 (8%)	5/50 (10%)	8/80 (10%)
Lung, alveolar epithelium	M	2/50 (4%)	9/50 (18%)	6/50 (12%)	13/49 (27%)	17/50 (34%)	12/73 (16%)
	F	5/50 (10%)	5/50 (10%)	3/50 (6%)	9/50 (18%)	11/50 (22%)	11/78 (14%)
Forestomach, epithelium	M	4/50 (8%)	3/50 (6%)	3/50 (6%)	5/48 (10%)	4/48 (18%)	40/72 (56%)
	F	4/50 (8%)	5/49 (10%)	4/47 (9%)	7/48 (15%)	14/50 (28%)	47/79 (59%)
Ovary, germinal epithelium	F	2/49 (4%)	3/49 (6%)	8/48 (17%)	15/50 (30%)	15/50 (30%)	19/79 (23%)
Ovary, granulosa cells	F	1/49 (2%)	0/49 (0%)	2/48 (4%)	3/50 (6%)	3/50 (6%)	2/79 (3%)
Mammary gland	F	2/50 (4%)	0/50 (0%)	2/50 (4%)	4/50 (8%)	7/50 (14%)	2/80 (3%)
Harderian gland	M	1/50 (2%)	3/49 (6%)	4/50 (8%)	6/47 (13%)	8/47 (17%)	5/40 (13%)
	F	1/50 (2%)	5/49 (10%)	9/48 (19%)	4/49 (8%)	4/49 (8%)	7/66 (11%)

Source: NTP, 1993.

Table 6-3. Incidence of hyperplasia in male B6C3F$_1$ mice exposed to 1,3-butadiene by inhalation in the stop-exposure study

Organ/tissue	Concentration (duration of exposure)				
	0 ppm	200 ppm (40 weeks)	312 ppm (52 weeks)	625 ppm (13 weeks)	625 ppm (26 weeks)
Heart, endothelium	0/50 (0%)	6/50 (12%)	3/50 (6%)	7/50 (14%)	7/50 (14%)
Lung, alveolar epithelium	2/50 (4%)	18/50 (36%)	14/50 (28%)	10/50 (20%)	11/50 (22%)
Forestomach, epithelium	4/50 (8%)	10/48 (21%)	20/48 (42%)	8/50 (16%)	15/50 (30%)
Harderian gland	1/50 (2%)	4/48 (8%)	6/48 (13%)	3/42 (7%)	7/36 (19%)

Source: NTP, 1993.

6.3. CARCINOGENICITY

The first NTP mouse inhalation study of 1,3-butadiene was terminated early due to induction of fatal neoplasms (NTP, 1984); therefore, a second study (NTP, 1993) was conducted to better characterize the exposure-response relationship for neoplasms and nonneoplastic lesions induced in mice by exposure to 1,3-butadiene for 2 years. The concentrations ranged from 100-fold lower (6.25 ppm) up to the lowest concentration (625 ppm) used in the first study. In addition, stop-exposure studies were conducted to assess the relationship between concentration and duration of exposure on the induction of neoplasms by 1,3-butadiene. Results of this study have also been published by Melnick et al. (1990a, b, c) and Melnick and Huff (1992). Miller and Boorman (1990) provided morphological descriptions of the neoplastic lesions induced in B6C3F$_1$ mice by 1,3-butadiene. The results are presented here in two parts, 2-year study and stop-exposure study.

6.3.1. 2-Year Study (NTP, 1993)

The details of the study design are described in Section 6.2. For neoplasms that were considered to be lethal tumors, the tumor incidence was analyzed using the life table test, a survival-adjusted procedure appropriate for rapidly lethal tumors (Cox, 1972; Tarone, 1975). For incidental tumors (tumors discovered as a result of death from an unrelated cause), the primary statistical method used was the logistic regression test. Alternate statistical methods included the Fisher exact test and the Cochran-Armitage trend test (Armitage, 1971; Gart et al., 1979), analyses based on the overall proportion of tumor-bearing animals. Tests of significance included pairwise comparisons of each dose group and a test for an overall concentration-response trend.

Exposure of male and female mice to 1,3-butadiene induced a variety of common and uncommon tumors at multiple sites. The incidences of primary neoplasms associated with exposure to 1,3-butadiene (for the 2-year study) are presented in Tables 6-4 and 6-5. The percentage of animals bearing malignant tumors increased from about 30% in the controls to nearly 90% in the highest exposure group, 625 ppm. The results of interim evaluations for 9 months and 15 months are presented in Tables 6-6 and 6-7.

As in the previous study (NTP, 1984), exposure of mice to 1,3-butadiene was associated with the development of malignant lymphocytic lymphomas and to a lesser extent with histiocytic sarcomas. The incidence of malignant lymphomas, particularly lymphocytic lymphomas, was significantly increased in males and females exposed to 625 ppm and in females exposed to 20 and 200 ppm (survival-adjusted) compared with controls. In addition, there were significant exposure-response trends ($p<0.001$) in both sexes. The lymphocytic lymphomas were well differentiated and occurred as early as week 23, peaking before the 15-month interim evaluation. Many organs, particularly the spleen, lymph nodes, liver, lung, and kidney, were affected in mice with lymphocytic lymphoma; however, the thymus was involved in most mice and was the primary organ affected in some. The lymphocytic lymphomas consisted of uniform populations of small- to medium-sized lymphocytes, whereas the mixed and undifferentiated lymphomas generally consisted of more heterogeneous populations of lymphocytes with pleomorphism and atypia. Other histological types of malignant lymphomas (mixed and undifferentiated), commonly associated with the spontaneous lymphomas in aging $B6C3F_1$ mice, were seen at low incidence in some groups. The incidences of histiocytic sarcoma were significantly increased in males and females exposed to 200 and 625 ppm and in males exposed to 62.5 ppm. The histiocytic sarcomas (previously referred to as reticulum cell sarcomas or type A sarcomas) were large and monomorphic, with dark basophilic nuclei and relatively abundant eosinophilic cytoplasm.

Hemangiosarcomas of the heart were observed in male (at $\geq$20 ppm) and female (at $\geq$62.5 ppm) mice exposed to 1,3-butadiene for 2 years. The incidences of hemangiosarcomas of the heart were significantly increased in male mice exposed to $\geq$62.5 ppm and in female mice exposed to $\geq$200 ppm. There was a significant exposure-response trend in both sexes. The cardiac hemangiosarcomas were observed in all ventricular locations, but were more frequent in

Table 6-4. Incidence of primary neoplasms in male B6C3F$_1$ mice exposed to 1,3-butadiene by inhalation for 103 weeks

Target organ	Neoplastic lesion	Concentration (ppm)					
		0	6.25	20	62.5	200	625
All organs	Malignant lymphoma (histiocytic, lymphocytic, mixed, NOS, or undifferentiated)	4/50 (8%)[a] 9.8%[b] *p*<0.001[c]	2/50 (4%) 5.1% *p*=0.302N	4/50 (8%) 12.2% *p*=0.528	6/50 (12%) 17.7% *p*=0.238	2/50 (4%) 4.0% *p*=0.627	51/73 (70%) 95.4% *p*<0.001
	Histiocytic sarcoma	0/50 (0%) 0.0% *p*<0.001[c]	0/50 (0%) 0.0%	4/50 (8%) 10.6% *p*=0.051	5/50 (10%) 14.3% *p*=0.021	7/50 (14%) 31.9% *p*<0.001	4/73 (5%) 10.8% *p*=0.043
	Malignant lymphoma or histiocytic sarcoma	4/50 (8%) 9.8% *p*<0.001[c]	2/50 (4%) 5.1% *p*=0.302N	8/50 (16%) 21.5% *p*=0.118	11/50 (22%) 29.6% *p*=0.022	9/50 (18%) 34.7% *p*=0.005	55/73 (75%) 95.9% *p*<0.001
Heart	Hemangiosarcoma	0/50 (0%) 0.0% *p*<0.001[c]	0/49 (0%) 0.0% NA	1/50 (2%) 3.4% *p*=0.451	5/48 (10%) 19.4% *p*=0.011	20/48 (42%) 93.3% *p*<0.001	4/73 (5%) 44.6% *p*<0.001
Lungs	Alveolar/bronchiolar adenoma	18/50 (36%) 46.9% *p*=0.200[d]	20/50 (40%) 47.3% *p*=0.517	10/50 (20%) 28.2% *p*=0.080N	25/49 (51%) 74.2% *p*=0.036	21/50 (42%) 100.0% *p*=0.061	3/73 (4%) 59.4% *p*=0.492
	Alveolar/bronchiolar carcinoma or adenocarcinoma	5/50 (10%) 14.3% *p*<0.001[c]	6/50 (12%) 15.4% *p*=0.577	11/50 (22%) 38.3% *p*=0.017	12/49 (24%) 42.9% *p*=0.006	22/50 (44%) 94.6% *p*<0.001	3/73 (4%) 59.4% *p*<0.001
	Alveolar/bronchiolar adenoma, adenocarcinoma, or carcinoma	21/50 (42%) 54.9% *p*<0.001[c]	23/50 (46%) 54.5% *p*=0.552N	19/50 (38%) 53.6% *p*=0.276	31/49 (63%) 87.9% *p*<0.001	35/50 (70%) 100.0% *p*<0.001	3/73 (4%) 59.4% *p*<0.001
Forestomach	Squamous cell papilloma	1/50 (2%) 2.5% *p*<0.001[d]	0/50 (0%) 0.0% *p*=0.535N	0/50 (0%) 0.0% *p*=0.486N	1/50 (2%) 4.5% *p*=0.739	7/50 (14%) 51.7% *p*=0.012	2/73 (3%) 40.0% *p*=0.446
	Squamous cell papilloma or carcinoma	1/50 (2%) 2.5% *p*<0.001[c]	0/50 (0%) 0.0% *p*=0.481N	0/50 (0%) 0.0% *p*=0.545N	1/50 (2%) 4.5% *p*=0.679	8/50 (16%) 54.5% *p*<0.001	4/73 (5%) 51.8% *p*<0.001
Liver	Hepatocellular adenoma	13/50 (26%) 32.1% *p*=0.042[d]	13/50 (26%) 31.3% *p*=0.552	19/50 (38%) 52.1% *p*=0.158	16/48 (33%) 57.0% *p*=0.261	23/48 (48%) 92.2% *p*=0.008	5/72 (7%) 100.0% *p*=0.253
	Hepatocellular carcinoma	11/50 (22%) 26.0% *p*=0.036[d]	16/50 (32%) 36.6% *p*=0.142	16/50 (32%) 44.8% *p*=0.389	17/48 (35%) 58.3% *p*=0.088	26/48 (54%) 100.0% *p*<0.001	1/72 (2%) 50.0% *p*=0.347

Table 6-4. Incidence of primary neoplasms in male B6C3F$_1$ mice exposed to 1,3-butadiene by inhalation for 103 weeks (continued)

Target organ	Neoplastic lesion	Concentration (ppm)					
		0	6.25	20	62.5	200	625
Liver (cont.)	Hepatocellular adenoma or carcinoma	21/50 (42%) 47.9% *p*=0.067[d]	23/50 (46%) 53.0% *p*=0.375	30/50 (60%) 70.1% *p*=0.078	25/48 (52%) 79.2% *p*=0.185	33/48 (69%) 100.0% *p*=0.030	5/72 (7%) 100.0% *p*=0.450
Harderian gland	Adenoma	6/50 (12%) 14.8% *p*<0.001[d]	7/50 (14%) 17.3% *p*=0.497	8/50 (16%) 25.8% *p*=0.395	19/50 (38%) 63.4% *p*<0.001	30/50 (60%) 95.4% *p*<0.001	6/73 (8%) 100.0% *p*=0.264
	Carcinoma	0/50 (0%) 0.0% *p*=0.720[d]	1/50 (2%) 2.6% *p*=0.522	1/50 (2%) 4.2% *p*=0.425	3/50 (6%) 11.7% *p*=0.086	2/50 (4%) 6.3% *p*=0.352	0/73 (0%) 0.0% NA
	Adenoma or carcinoma	6/50 (12%) 14.8% *p*<0.001[d]	7/50 (14%) 17.3% *p*=0.497	9/50 (18%) 29.5% *p*=0.217	20/50 (40%) 64.9% *p*<0.001	31/50 (62%) 95.5% *p*<0.001	6/73 (8%) 100.0% *p*=0.002
Preputial gland	Carcinoma	0/50 (0%) 0.0% *p*<0.001[c]	0/50 (0%) 0.0% NA	0/50 (0%) 0.0% NA	0/50 (0%) 0.0% NA	5/50 (10%) 45.7% *p*<0.001	0/73 (0%) 0.0% NA

[a]Overall rate: number of tumor-bearing animals/number of animals examined.
[b]Survival-adjusted rate. Kaplan-Meier estimated neoplasm incidence at the end of the study after adjustment for intercurrent mortality.
[c]Life table test. Beneath the control incidence are the p values associated with the trend test. Beneath the dosed group incidence are the P values corresponding to pairwise comparison between the control and dosed groups. The life table analysis regards neoplasms in animals dying prior to terminal kill as being (directly or indirectly) the cause of death.
[d]Logistic regression test. This test regards the neoplasms as nonfatal.

NA = not applicable; no tumors in these groups.
NOS = not otherwise specified.
N = incidence in dose group is lower than in control group.
Source: NTP, 1993.

Table 6-5. Incidence of primary neoplasms in female B6C3F$_1$ mice exposed to 1,3-butadiene by inhalation for 103 weeks

Target organ	Neoplastic lesion	Concentration (ppm)					
		0	6.25	20	62.5	200	625
All organs	Malignant lymphoma (lymphocytic, mixed, NOS, or undifferentiated cell type)	6/50 (12%)[a] 14.6%[b] *p*<0.001[c]	12/50 (24%) 34% *p*=0.068	11/50 (22%) 38.7% *p*=0.029	7/50 (14%) 35.9% *p*=0.055	9/50 (18%) 39.7% *p*<0.001	32/80 (40%) 70.8% *p*<0.001
	Histiocytic sarcoma	3/50 (6%) 6.9% *p*<0.001[c]	2/50 (4%) 4.5% *p*=0.518N	7/50 (14%) 20.0% *p*=0.077	4/50 (8%) 17.7% *p*=0.195	7/50 (14%) 28.1% *p*=0.002	4/80 (5%) 10.3% *p*=0.038
	Malignant lymphoma or histiocytic sarcoma	9/50 (18%) 20.5% *p*<0.001[c]	14/50 (28%) 37.0% *p*=0.136	18/50 (36%) 52.1% *p*=0.005	11/50 (22%) 47.2% *p*=0.021	16/50 (32%) 56.7% *p*<0.001	36/80 (45%) 73.9% *p*<0.001
Heart	Hemangiosarcoma	0/50 (0%) 0.0% *p*<0.001[c]	0/50 (0%) 0.0% NA	0/50 (0%) 0.0% NA	1/49 (2%) 4.8% *p*=0.392	21/50 (42%) 100.0% *p*<0.001	23/80 (29%) 100.0% *p*<0.001
Lungs	Alveolar/bronchiolar adenoma	4/50 (8%) 10.5% *p*=0.002[d]	11/50 (22%) 30.9% *p*=0.039	12/50 (24%) 40.7% *p*=0.013	17/50 (34%) 64.8% *p*<0.001	14/49 (29%) 100.0% *p*=0.002	17/78 (22%) 100.0% *p*=0.010
	Alveolar/bronchiolar adenocarcinoma or carcinoma	0/50 (0%) 0.0% *p*<0.001[c]	5/50 (10%) 13.3% *p*=0.029	11/50 (22%) 42.9% *p*<0.001	9/50 (18%) 40.8% *p*<0.001	19/49 (39%) 100.0% *p*<0.001	8/78 (10%) 100.0% *p*<0.001
	Alveolar/bronchiolar adenoma, adenocarcinoma, or carcinoma	4/50 (8%) 10.5% *p*<0.001[c]	15/50 (30%) 39.5% *p*=0.004	19/50 (38%) 63.7% *p*<0.001	24/50 (48%) 78.5% *p*<0.001	25/49 (51%) 100.0% *p*<0.001	22/78 (28%) 100.0% *p*<0.001
Forestomach	Squamous cell papilloma	0/50 (0%) 0.0% *p*<0.001[d]	0/50 (0%) 0.0% NA	2/50 (4%) 8.3% *p*=0.149	1/50 (2%) 9.1% *p*=0.260	3/50 (6%) 100.0% *p*=0.078	16/80 (20%) 100.0% *p*=0.002
	Squamous cell carcinoma	0/50 (0%) 0.0% *p*<0.001[c]	0/50 (0%) 0.0% NA	1/50 (2%) 4.2% *p*=0.414	1/50 (2%) 8.3% *p*=0.277	1/50 (2%) 3.8% *p*=0.374	6/80 (8%) 70.5% *p*<0.001
	Squamous cell papilloma or carcinoma	0/50 (0%) 0.0% *p*<0.001[c]	0/50 (0%) 0.0% NA	3/50 (6%) 12.5% *p*=0.056	2/50 (4%) 16.7% *p*=0.044	4/50 (8%) 100.0% *p*=0.001	22/80 (28%) 100.0% *p*<0.001
Liver	Hepatocellular adenoma	11/49 (22%) 29.7% *p*=0.599N	10/49 (20%) 27.8% *p*=0.531N	9/50 (18%) 30.3% *p*=0.519N	14/48 (28%) 65.8% *p*=0.025	15/50 (29%) 89.0% *p*=0.009	1/80 (1%) 100.0% *p*=0.505
	Hepatocellular carcinoma	4/49 (8%) 10.3% *p*=0.178[d]	6/49 (12%) 14.5% *p*=0.381	8/50 (16%) 25.0% *p*=0.141	9/50 (18%) 39.9% *p*=0.066	8/50 (16%) 82.7% *p*=0.006	1/80 (1%) 12.5% *p*=0.910

Table 6-5. Incidence of primary neoplasms in female B6C3F$_1$ mice exposed to 1,3-butadiene by inhalation for 103 weeks (continued)

Target organ	Neoplastic lesion	Concentration (ppm)					
		0	6.25	20	62.5	200	625
Liver (cont.)	Hepatocellular adenoma or carcinoma	15/49 (31%) 39.3% *p*=0.497[d]	14/49 (29%) 34.3% *p*=0.504N	15/50 (30%) 45.5% *p*=0.441	19/50 (38%) 74.8% *p*=0.027	16/50 (32%) 91.7% *p*=0.008	2/80 (3%) 100.0% *p*=0.302
Ovary	Benign granulosa cell tumor	1/49 (2%) 2.8% *p*=0.030[d]	0/49 (0%) 0.0% *p*=0.517N	1/48 (2%) 3.2% *p*=0.735	6/50 (12%) 28.5% *p*=0.026	6/50 (12%) 100.0% *p*=0.020	6/79 (8%) 27.1% *p*=0.303
	Malignant granulosa cell tumor	0/49 (0%) 0.0% *p*=0.068[d]	0/49 (0%) 0.0% NA	0/48 (0%) 0.0% NA	3/50 (6%) 19.3% *p*=0.046	2/50 (4%) 54.2% *p*=0.037	0/79 (0%) 0.0% NA
	Benign or malignant granulosa cell tumor	1/49 (2%) 2.8% *p*=0.006[d]	0/49 (0%) 0.0% *p*=0.517N	1/48 (2%) 3.2% *p*=0.735	9/50 (18%) 42.9% *p*=0.001	8/50 (16%) 100.0% *p*=0.001	6/79 (8%) 27.1% *p*=0.303
Mammary gland	Adenoacanthoma	0/50 (0%) 0.0% *p*=0.025[c]	1/50 (2%) 2.9% *p*=0.489	2/50 (4%) 7.7% *p*=0.152	6/50 (12%) 32.5% *p*<0.001	4/50 (8%) 13.6% *p*=.021	0/80 (0%) 0.0% NA
	Carcinoma	0/50 (0%) 0.0% *p*<0.001[c]	2/50 (4%) 5.8% *p*=0.221	2/50 (4%) 5.7% *p*=0.192	6/50 (12%) 16.2% *p*=0.008	11/50 (22%) 39.1% *p*<0.001	12/80 (15%) 100.0% *p*<0.001
	Malignant mixed tumor	0/50 (0%) 0.0% *p*<0.001[c]	0/50 (0%) 0.0% NA	0/50 (0%) 0.0% NA	0/50 (0%) 0.0% NA	0/50 (0%) 0.0% NA	4/80 (5%) 29.4% *p*=0.003
Harderian gland	Adenoma	8/50 (16%) 20.8% *p*=0.046[d]	10/50 (20%) 29.2% *p*=0.356	6/50 (12%) 20.7% *p*=0.511N	15/50 (30%) 61.0% *p*=0.016	20/50 (40%) 89.3% *p*=0.001	9/80 (11%) 45.2% *p*=0.176
	Carcinoma	0/50 (0%) 0.0% *p*=0.873N[d]	1/50 (2%) 2.7% *p*=0.493	1/50 (2%) 2.3% *p*=0.631	0/50 (0%) 0.0% NA	1/50 (2%) 50.0% *p*=0.085	0/80 (0%) 0.0% NA
	Adenoma or carcinoma	8/50 (16%) 20.8% *p*=0.061[d]	10/50 (20%) 29.2% *p*=0.356	7/50 (14%) 22.5% *p*=0.575N	15/50 (30%) 61.0% *p*=0.016	20/50 (40%) 89.3% *p*=0.001	9/80 (11%) 45.2% *p*=0.176

[a]Overall rate; number of tumor-bearing animals/number of animals examined.
[b]Survival-adjusted rate. Kaplan-Meier estimated neoplasm incidence at the end of the study after adjustment for intercurrent mortality.
[c]Life table test. Beneath the control incidence are the p values associated with the trend test. Beneath the dosed group incidence are the p values corresponding to pairwise comparison between the control and dosed groups. The life table analysis regards neoplasm in animals dying prior to terminal kill as being (directly or indirectly) the cause of death.
[d]Logistic regression test. This test regards the neoplasms as nonfatal.

NA = not applicable; no tumors in these groups.
NOS = not otherwise specified.
N = incidence in dose group is lower than in control group.
Source: NTP, 1993.

Table 6-6. Incidence of primary neoplasms in male B6C3F$_1$ mice exposed to 1,3-butadiene by inhalation for 9 months and 15 months

Target organ	Neoplastic lesion		Concentration (ppm)					
			0	6.25	20	62.5	200	625
All organs	Malignant lymphoma (histiocytic, lymphocytic, mixed, NOS, or undifferentiated)	9 months						1/10
		15 months						2/7
Heart	Hemangiosarcoma	9 months						
		15 months					1/10	3/7
Lungs	Alveolar/ bronchiolar adenoma, adenocarcinoma, or carcinoma	9 months	1/10	1/1	½	0/10	2/10	3/10
		15 months				2/10	4/10	5/7
Forestomach	Squamous cell papilloma or carcinoma	9 months						1/10
		15 months					1/10	3/7
Liver	Hepatocellular adenoma or carcinoma	9 months	4/10	0/10	1/10	0/10	1/10	1/10
		15 months	2/10	1/10	4/10	3/10	4/10	5/7
Harderian gland	Adenoma or carcinoma	9 months						
		15 months			2/10	4/10	3/10	3/7

[a]Overall rate: number of tumor-bearing animals/number of animals examined.
[b]Survival-adjusted rate. Kaplan-Meier estimated neoplasm incidence at the end of the study after adjustment for intercurrent mortality.
[c]Life table test. Beneath the control incidence are the p values associated with the trend test. Beneath the dosed group incidence are the p values corresponding to pairwise comparison between the control and dosed groups. The life table analysis regards neoplasms in animals dying prior to terminal kill as being (directly or indirectly) the cause of death.
[d]Logistic regression test. This test regards the neoplasms as nonfatal.

NA = not applicable; no tumors in these groups.
NOS = not otherwise specified.
N = incidence in dose group is lower than in control group.

Source: NTP, 1993.

Table 6-7. Incidence of primary neoplasms in female B6C3F$_1$ mice exposed to 1,3-butadiene by inhalation for 9 months and 15 months

Target organ	Neoplastic lesion		Concentration (ppm)					
			0	6.25	20	62.5	200	625
All organs	Malignant lymphoma (lymphocytic, mixed, NOS, or undifferentiated cell type)	9 months						1/8
		15 months	1/10				1/10	0/2
Heart	Hemangiosarcoma	9 months						
		15 months					1/10	2/2
Lungs	Alveolar/bronchiolar adenoma, adenocarcinoma, or carcinoma	9 months					2/10	1/8
		15 months				3/10	3/10	½
Forestomach	Squamous cell papilloma or carcinoma	9 months						
		15 months				1/10	2/10	½
Liver	Hepatocellular adenoma or carcinoma	9 months						
		15 months	1/10	1/10	0/10	1/10	3/10	½
Ovary	Benign or malignant granulosa cell tumor	9 months					1/10	
		15 months				1/10	4/10	½
Mammary gland	Adenoacanthoma, adenocarcinoma, carcinoma, or malignant mixed tumor	9 months						
		15 months					2/10	½
Harderian gland	Adenoma or carcinoma	9 months						1/5
		15 months	2/9	1/1	1/1	1/10	3/10	0/2

[a]Overall rate; number of tumor-bearing animals/number of animals examined.
[b]Survival-adjusted rate. Kaplan-Meier estimated neoplasm incidence at the end of the study after adjustment for intercurrent mortality.
[c]Life table test. Beneath the control incidence are the p values associated with the trend test. Beneath the dosed group incidence are the p values corresponding to pairwise comparison between the control and dosed groups. The life table analysis regards neoplasm in animals dying prior to terminal kill as being (directly or indirectly) the cause of death.
[d]Logistic regression test. This test regards the neoplasms as nonfatal.

NA = not applicable; no tumors in these groups.
NOS = not otherwise specified.
N = incidence in dose group is lower than in control group.

Source: NTP, 1993.

the left ventricular wall. Typical hemangiosarcomas had solid foci of anaplastic, pleomorphic spindle cells at the center with a loose arrangement at the periphery. They were occasionally multifocal and frequently coexisted with foci of endothelial hyperplasia distant and separate from the main neoplasm. Hemangiosarcomas of the heart are considered uncommon in untreated $B6C3F_1$ mice (none were observed in 573 male and in 558 female historical controls in NTP inhalation studies). In male mice, the lower incidence of cardiac hemangiosarcoma at 625 ppm compared with that at 200 ppm, was attributed to the early mortality due to induction of lethal lymphocytic lymphoma at 625 ppm. The time-to-tumor detection for all hemangiosarcomas of the heart ranged from 682 days at 20 ppm to 289 days at 625 ppm for males and from 649 days at 20 ppm to 307 days at 625 ppm for females. When hemangiosarcomas occurred in multiple organs, the cardiac neoplasms were usually designated as primary, because the incidence of hemangiosarcomas was highest in the heart and the earliest lesions occurred in the heart. However, it could not be determined with certainty if the hemangiosarcomas observed in other organs were metastases or primary neoplasms. Subcutaneous, splenic, and hepatic hemangiosarcomas that were found in the absence of cardiac hemangiosarcomas may reflect the development of spontaneous vascular neoplasms known to occur in $B6C3F_1$ mice.

Exposure of mice to 1,3-butadiene was also associated with an increased incidence of pulmonary neoplasms in male and female mice. Although the incidence of alveolar/bronchiolar adenomas was not significantly increased in male mice in the 2-year study, the combined incidences of alveolar/bronchiolar adenocarcinomas and carcinomas and the combined incidences of the benign and malignant pulmonary neoplasms were significantly increased at 62.5, 200, and 625 ppm. In female mice, the incidences of the benign and malignant neoplasms analyzed separately or together were significantly increased in all exposure groups compared with controls. Thus, even at 6.25 ppm, 1,3-butadiene was carcinogenic to female $B6C3F_1$ mice. The lower incidence of lung neoplasms at 625 ppm compared with the incidence at 200 ppm was attributed to the high rate of early deaths due to the competing risks of lymphocytic lymphoma in female mice exposed to 625 ppm. There was a significant exposure-response trend for combined adenomas and carcinomas in both sexes. The time-to-tumor detection for lung tumors combined ranged from 587 days at 6.25 ppm to 251 days at 625 ppm for males, and from 519 days at 6.25 ppm to 275 days at 625 ppm for females. The spectrum of lung lesions ranged from alveolar epithelial hyperplasia (Section 3.2 of this chapter) to adenomas, carcinomas, and adenocarcinomas. Histologically, the alveolar/bronchiolar adenomas exhibited distortion of the alveolar structure due to the formation of complex, irregular papillary patterns; the alveolar/bronchiolar carcinomas were similar, but consisted of heterogeneous cell populations with various degrees of cellular pleomorphism and atypia. The adenocarcinomas were larger, highly anaplastic neoplasms, often accompanied by hemorrhage or necrosis.

In the forestomach, significant increases in squamous cell papillomas and carcinomas combined were observed in male mice exposed to $\geq$200 ppm and in female mice exposed to $\geq$62.5 ppm compared with controls. There was a significant exposure-response trend for papillomas and carcinomas combined in both sexes. The combined incidence of squamous cell papillomas and carcinomas of the forestomach (males, 4/575 [0.7%]; females, 9/561 [1.6%]) for historical controls suggests that these lesions are relatively uncommon in $B6C3F_1$ mice.

Increased incidences of hepatocellular adenomas and carcinomas were also seen in 1,3-butadiene-exposed mice (Tables 6-4 and 6-5). The hepatocellular adenomas were discrete, expansile masses; the carcinomas were larger than the adenomas and consisted of markedly disorganized hepatocytes. The low incidence of liver neoplasms observed in males and females at 625 ppm

probably reflects increased early deaths from malignant lymphoma. Hepatocellular adenomas and carcinomas are common neoplasms in $B6C3F_1$ mice, occurring in 196/572 (34%) of male and 87/558 (15.6%) of female historical controls in NTP inhalation studies. The data suggest that 1,3-butadiene has only a weak tumorigenic effect in the livers of male and female mice. However, a chemical-related effect is supported by the detection of an activated K-ras oncogene in liver neoplasms obtained from mice exposed to 1,3-butadiene (Goodrow et al., 1990). According to Reynolds et al. (1987), activated K-ras oncogene had never been detected in liver neoplasms from untreated $B6C3F_1$ mice.

Although a variety of neoplasms were seen in the ovaries of female mice, only benign and malignant granulosa cell tumors were definitely attributed to exposure to 1,3-butadiene (Table 6-5). The ovarian granulosa cell tumors varied from small benign tumors to large cystic tumors with thick trabeculae and spaces filled with blood or clear fluid. The overall historical control incidence at NTP for benign and malignant granulosa cell tumors each was 1/548 (0.2%).

Increased incidences of mammary gland neoplasms were seen in female mice exposed to $\geq$62.5 ppm 1,3-butadiene. Mammary tumors included adenoacanthomas, adenocarcinomas, and malignant mixed tumors, the latter occurring only at 625 ppm. The mammary gland tumors combined exhibited a significant exposure-response relationship. The adenoacanthomas were considered variants of adenocarcinomas that have prominent squamous differentiation. The malignant mixed tumors consisted of epithelial components arranged in glandlike structures and anaplastic spindle-cell components. Mammary gland adenocarcinomas and adenoacanthomas were considered uncommon in female $B6C3F_1$ mice; the overall historical incidence at NTP was 21/561 (3.7%) for carcinomas and 1/561 (0.2%) for adenoacanthomas in female control mice.

The Harderian gland was identified as another site of 1,3-butadiene-induced neoplasms in male and female mice (Tables 6-4 and 6-5), with significant exposure-related increases in adenomas at 62.5 and 200 ppm and a low incidence of carcinomas in males exposed to 6.25, 20, 62.5, or 200 ppm. The low incidence of Harderian gland tumors at 625 ppm was attributed to early deaths due to lymphocytic lymphoma which precluded the development of Harderian gland tumors. The investigators noted that the occurrence of Harderian gland carcinomas in mice, particularly males, is unusual. The overall incidence of Harderian gland carcinomas was 2/575 (0.3%) in male and 3/561 (0.5%) in female historical controls at NTP. The 2-year historical incidence of adenomas and carcinomas (combined) of the Harderian gland for control groups in NTP inhalation studies was 25/575 (4.3%) for males and 13/561 (2.3%) for females.

Preputial gland carcinomas, also considered to be rare neoplasms in $B6C3F_1$ mice, were seen in five males ($p<0.05$) exposed to 200 ppm (none were reported in one survey of NTP historic control data). These tumors were also thought to be exposure-related lesions. Some preputial carcinomas were composed of large eosinophilic epithelial cells that were well differentiated; more frequently, the carcinomas had necrotic cores and a thin layer of very anaplastic basophilic epithelial cells that aggressively invaded surrounding tissue and blood vessels.

Renal tubule adenomas were seen in 2/50 females exposed to 200 ppm 1,3-butadiene and in 1/50, 3/48, and 1/49 of males exposed to 6.25, 62.5, and 200 ppm, respectively. At the 15-month evaluation, renal tubular adenoma occurred in 1/7 males exposed to 625 ppm. The historical incidence of spontaneous renal tubule adenomas in untreated control groups in NTP inhalation studies was 1/571 (0.2%) for males and 0/559 (0.0%) for females. Histologically, the renal tubule adenomas contained multiple dilated tubules separated by thin connective tissue septa. These renal lesions were probably related to exposure to 1,3-butadiene in males and possibly related to exposure in females.

One neurofibrosarcoma of the subcutaneous tissue was observed in two females exposed to 625 ppm at the 15-month evaluation. In the 2-year study, the combined incidences of neurofibrosarcomas and sarcomas of the subcutaneous tissue were significantly increased in female mice exposed to 62.5 ppm (p=0.017), 200 ppm (p=0.002), and 625 ppm (p=0.013) by the life table test. Subcutaneous tissue sarcomas (all types) were considered uncommon spontaneous neoplasms; the historical incidence was 2/561 (0.4%) for female controls at NTP, suggesting that these subcutaneous tissue neoplasms may have been exposure-related. The historical incidence for NTP inhalation studies was not reported.

One adenoma and one carcinoma of the Zymbal's gland were seen in females exposed to 625 ppm; one adenoma also occurred in a concurrent control male mouse, but none were reported in historical controls. The report indicated that these Zymbal's gland neoplasms may be related to 1,3-butadiene exposure.

Carcinomas of the small intestine, another uncommon tumor in the $B6C3F_1$ mouse, were seen in two females exposed to 6.25 ppm and in one female exposed to 62.5 ppm. One carcinoma each was seen in one male each exposed to 6.25, 20, or 62.5 ppm, and in two males exposed to 200 ppm. The relationship of these neoplasms to exposure to 1,3-butadiene could not be determined; however, controls did not exhibit proliferative lesions of the intestine.

In supplemental analyses, the authors performed a "Poly-3" quantal response test (Bailer and Portier, 1988; Portier and Bailer, 1989) as an alternative to the logistic regression analyses, whose sensitivity was reduced by the decreased survival in the higher exposure groups. For tumor sites related to butadiene exposure, the "Poly-3" test detected significant responses in some of the exposure groups that had not been detected by the logistic regression analyses. The overall results were consistent with those already presented.

The authors also fitted a modified Weibull model (Portier et al., 1986) to the "Poly-3" survival-adjusted tumor rates to determine the shape parameters for the exposure-response relationships. About half of the tumor sites associated with butadiene exposure had exposure-response relationships consistent with a linear model (i.e., shape parameter of 1). Most of the other tumor sites exhibited supralinear exposure-response relationships (i.e., steep slope in low-exposure region; shape parameter significantly <1). These sites were the liver in male mice, the mammary gland in females, and the Harderian gland and lung in both sexes. Only the malignant lymphoma in males and heart hemangiosarcoma in females had a shape parameter significantly greater than 1, suggestive of a sublinear exposure-response relationship.

6.3.2. 2-Year Stop-Exposure Study (NTP, 1993)

An additional study with $B6C3F_1$ mice, referred to as "stop-exposure study" was conducted to assess the relationship between exposure level and duration of exposure to outcome of 1,3-butadiene carcinogenicity. Groups of 50 male mice were exposed 6 hours/day, 5 days/week at concentrations of (a) 200 ppm for 40 weeks, (b) 625 ppm for 13 weeks, (c) 312 ppm for 52 weeks, or (d) 625 ppm for 26 weeks. After the exposures were stopped, the animals were placed in control chambers for the remainder of the 103-week studies. The total exposures to 1,3-butadiene (concentration × duration of exposure) were approximately 8,000 ppm·weeks for groups exposed to 200 ppm for 40 weeks or 625 ppm for 13 weeks; the total exposures were approximately 16,000 ppm·week for groups exposed to 312 ppm for 52 weeks or 625 ppm for 26 weeks. No additional controls were included for these studies, because they were run concurrently with the 2-year studies.

Using the stop-exposure protocol, inhalation exposure to 1,3-butadiene had no effect on mean body weights. However, exposure to 1,3-butadiene markedly reduced survival in all stop-exposure groups as a result of the development of neoplasms, particularly malignant lymphomas and hemangiosarcomas of the heart (Figure 6-3). A comparison of the two groups receiving total exposures of 8,000 ppm·weeks showed that the survival of mice exposed to 625 ppm (13 weeks) was similar to that of mice exposed to 200 ppm (40 weeks). By contrast, the groups exposed to 16,000 ppm·weeks, survival of mice exposed to 625 ppm (26 weeks) was significantly lower than that of mice exposed to 312 ppm (52 weeks).

Neoplasms induced in the stop-exposure studies are summarized in Table 6-8. Overall, the data show that exposure of male mice to 1,3-butadiene using the stop-exposure protocol induced neoplasms at the same sites as those observed in the 2-year study.

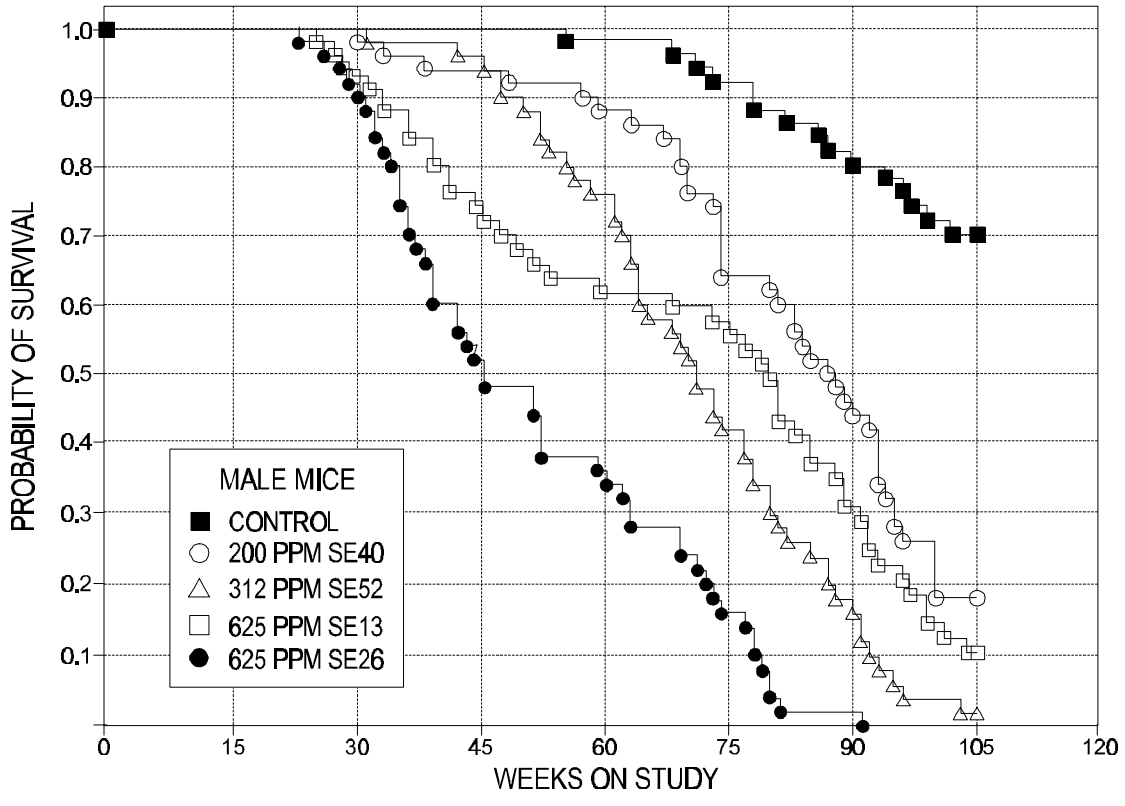

Figure 6-3. Kaplan-Meier survival curves for male mice in the stop-exposure inhalation study of 1,3-butadiene.
Source: NTP, 1993.

Table 6-8. Incidence of primary neoplasms in male B6C3F$_1$ mice exposed to 1,3-butadiene by inhalation in the stop-exposure study

Parameters		Concentration (duration of exposure)				
		0 ppm	200 ppm	625 ppm	312 ppm	625 ppm
Duration of exposures (weeks)		**103**	**40**	**13**	**52**	**26**
Total exposure (ppm ·weeks)		**0**	**8,000**	**8,000**	**16,000**	**16,000**
Target organ	**Neoplastic lesion**					
Hematopoietic	Lymphocytic malignant lymphoma	2/50[a] (4%) 4.7%[b] --	6/50 (12%) 26.7% *p*=0.033[c]	17/50 (34%) 35.8% *p*<0.001	4/50 (8%) 100.0% *p*=0.034	30/50 (60%) 81.5% *p*<0.001
	Lymphoma (mixed or NOS)	2/50 (4%) 5.3% --	2/50 (4%) 7.8% *p*=0.382[c]	5/50 (10%) 34.8% *p*=0.010	4/50 (8%) 58.0% *p*=0.005	3/50 (6%) 43.3% *p*=0.002
	Histiocytic sarcoma	0/50 (0%) 0.0% --	5/50 (10%) 21.3% *p*=0.006[c]	2/50 (4%) 28.9% *p*=0.011	7/50 (14%) 43.0% *p*<0.001	2/50 (4%) 15.6% *p*=0.036
	Malignant lymphoma or histiocytic sarcoma	4/50 (8%) 9.8% --	13/50 (26%) 46.8% *p*<0.001[c]	24/50 (48%) 72.1% *p*<0.001	15/50 (30%) 100.0% *p*<0.001	35/50 (70%) 91.2% *p*<0.001
	Malignant lymphoma (lymphocytic, mixed, or NOS)	4/50 (8%) 9.8% --	8/50 (16%) 32.4% *p*=0.023[c]	22/50 (44%) 58.2% *p*<0.001	8/50 (16%) 100.0% *p*<0.001	33/50 (66%) 89.5% *p*<0.001
Heart	Hemangiosarcoma	0/50 (0%) 0.0% --	15/50 (30%) 76.2% *p*<0.001[c]	7/50 (14%) 61.8% *p*<0.001	33/50 (66%) 100.0% *p*<0.001	13/50 (26%) 100.0% *p*<0.001
Lungs	Alveolar/bronchiolar adenoma	18/50 (36%) 46.9% --	24/50 (48%) 94.3% *p*=0.015[d]	17/50 (34%) 85.3% *p*=0.044	26/50 (52%) 100.0% *p*=0.001	12/50 (24%) 100.0% *p*<0.001
	Alveolar/bronchiolar adenocarcinoma or carcinoma	5/50 (10%) 14.3% --	22/50 (44%) 89.5% *p*<0.001[c]	18/50 (36%) 87.7% *p*<0.001	16/50 (32%) 100.0% *p*<0.001	11/50 (22%) 100.0% *p*<0.001

Table 6-8. Incidence of primary neoplasms in male B6C3F$_1$ mice exposed to 1,3-butadiene by inhalation in the stop-exposure study (continued)

Parameters		Concentration (duration of exposure)				
		0 ppm	200 ppm	625 ppm	312 ppm	625 ppm
Duration of exposures (weeks)		103	40	13	52	26
Total exposure (ppm ·weeks)		0	8,000	8,000	16,000	16,000
Target organ	Neoplastic lesion					
	Alveolar/bronchiolar adenoma, adenocarcinoma, or carcinoma	21/50 (42%) 54.9% --	36/50 (72%) 100.0% $p<0.001$[c]	28/50 (56%) 100.0% $p<0.001$	32/50 (64%) 100.0% $p<0.001$	17/50 (34%) 100.0% $p<0.001$
Liver	Hepatocellular adenoma	13/50 (26%) 32.1% --	27/49 (55%) 91.1% $p<0.001$[d]	19/49 (39%) 91.1% $p=0.042$	19/50 (38%) 100.0% $p=0.045$	11/50 (22%) 100.0% $p=0.284$
	Hepatocellular carcinoma	11/50 (22%) 26.0% --	14/49 (29%) 50.3% $p=0.530$[d]	14/49 (29%) 90.9% $p=0.142$	10/50 (20%) 74.6% $p=0.453$	4/50 (8%) 50.5% $p=0.393$
	Hepatocellular adenoma or carcinoma	21/50 (42%) 47.9% --	33/49 (67%) 93.4% $p=0.004$[d]	24/49 (49%) 94.4% $p=0.063$	24/50 (48%) 100.0% $p=0.169$	13/50 (26%) 100.0% $p=0.561$
Forestomach	Squamous cell papilloma	1/50 (2%) 2.5% --	3/50 (6%) 21.4% $p=0.195$[d]	4/50 (8%) 28.3% $p=0.260$	4/50 (8%) 100.0% $p=0.181$	4/50 (8%) 20.1% $p=0.301$
	Squamous cell carcinoma	0/50 (0%) 0.0% --	0/50 (0%) 0.0% NA	4/50 (8%) 51.6% $p<0.001$[d]	5/50 (10%) 33.1% $p<0.001$	6/50 (12%) 40.9% $p<0.001$
	Squamous cell papilloma or carcinoma	1/50 (2%) 2.5% --	3/50 (6%) 21.4% p=0.065[c]	7/50 (14%) 56.6% p<0.001	9/50 (18%) 100.0% p<0.001	10/50 (20%) 52.8% p<0.001
Harderian gland	Adenoma	6/50 (12%) 14.8% --	26/50 (52%) 87.9% p<0.001[d]	20/50 (40%) 94.3% p=0.001	28/50 (56%) 100.0% p<0.001	13/50 (26%) 100.0% p=0.046

Table 6-8. Incidence of primary neoplasms in male B6C3F$_1$ mice exposed to 1,3-butadiene by inhalation in the stop-exposure study (continued)

Parameters		Concentration (duration of exposure)				
		0 ppm	200 ppm	625 ppm	312 ppm	625 ppm
Duration of exposures (weeks)		103	40	13	52	26
Total exposure (ppm ·weeks)		0	8,000	8,000	16,000	16,000
Target organ	Neoplastic lesion					
	Carcinoma	0/50 (0%) 0.0% --	2/50 (4%) 5.6% p=0.397[d]	4/50 (8%) 38.8% p=0.190	2/50 (4%) 51.5% p=0.006	0/50 (0%) 0.0% NA
	Adenoma or carcinoma	6/50 (12%) 14.8% --	27/50 (54%) 88.3% p<0.001[d]	23/50 (46%) 100.0% p<0.001	30/50 (60%) 100.0% p<0.001	13/50 (26%) 100.0% p=0.046
Kidney	Renal tubule adenoma	0/50 (0%) 0.0% --	4/48 (8%) 17.4% *p*=0.073[d]	1/50 (2%) 14.3% *p*=0.273	3/49 (6%) 27.8% *p*=0.075	1/50 (2%) 6.3% *p*=0.731
Brain[e]	Malignant glioma	0/50 (0%)	0/50 (0%)	2/50 (4%)	0/50 (0%)	1/50 (2%)
	Malignant neuroblastomas	0/50 (0%)	0/50 (0%)	2/50 (4%)	0/50 (0%)	0/50 (0%)
Preputial gland	Carcinoma	0/50 (0%) 0.0% --	1/50 (2%) 10.0% *p*=0.368[c]	4/50 (8%) 16.9% *p*=0.039	4/50 (8%) 100.0% *p*<0.001	3/50 (6%) 100.0% *p*=0.002
	Adenoma or carcinoma	0/50 (0%) 0.0% --	1/50 (2%) 10.0% *p*=0.368[c]	5/50 (10%) 22.9% *p*=0.013	4/50 (8%) 100.0% *p*<0.001	3/50 (6%) 100.0% *p*=0.002
Zymbal's gland	Adenoma or carcinoma	1/50 (2%) 2.9% --	1/50 (2%) 4.8% *p*=0.531[c]	2/50 (4%) 8.8% *p*=0.178	0/50 (0%) 0.0% *p*=0.998N	2/50 (4%) 37.3% *p*=0.009

Table 6-8. Incidence of primary neoplasms in male B6C3F$_1$ mice exposed to 1,3-butadiene by inhalation in the stop-exposure study (continued)

[a]Overall rate, number of tumor-bearing animals/number of animals examined.
[b]Survival-adjusted rate. Kaplan-Meier estimated neoplasm incidence at the end of the study after adjustment for intercurrent mortality.
[c]Life table test. The p values for pairwise comparison of exposed groups with controls are beneath the exposed group incidence. N refers to negative association with control group. The life table analysis regards neoplasms in animals dying prior to terminal kill as being (directly or indirectly) the cause of death.
[d]Logistic regression test. This test regards the neoplasms as nonfatal.
[e]No statistical analysis.

NA = not applicable.
NOS = not otherwise specified.
N = incidence in dose group is lower than in control group.

Source: NTP, 1993a.

Lymphocytic lymphomas of thymic origin occurred at a markedly increased incidence in mice exposed to 625 ppm for 13 or 26 weeks. According to the life table test, the incidence of lymphocytic lymphoma was also significantly increased in mice exposed to 200 ppm for 40 weeks or 312 ppm for 52 weeks. The incidence of histiocytic sarcomas was significantly increased (life table test) in mice in all stop-exposure groups as well.

The lower incidences of lymphocytic lymphomas at 200 ppm (40 weeks) and 312 ppm (52 weeks) compared to 625 ppm for 13 and 26 weeks, respectively, demonstrate that the concentration of 1,3-butadiene is a greater contributing factor in the development of this lesion than the duration of exposure, i.e., a high concentration for a short duration is more effective than a lower concentration of longer duration. A comparison of the 200 ppm (40 weeks) versus the 625 ppm (13 weeks) and of the 312 ppm (52 weeks) versus the 625 ppm (26 weeks) lymphocytic lymphoma results using a life table test confirms that the higher concentration/shorter duration regimen is significantly more effective than the lower concentration/longer duration regimen within each cumulative exposure grouping (p=0.005 for 8,000 ppm·weeks and p<0.001 for 16,000 ppm·weeks) after survival differences are taken into account.

As observed in the 2-year study, lymphocytic lymphomas occurred very early after exposure started: as early as 23 weeks in the group exposed to 625 ppm for 26 weeks and as early as 24 weeks in the group exposed to 625 ppm for 13 weeks. This lesion accounted for 24 and 17, respectively, of the first 25 deaths occurring in these groups by weeks 45 and 79, respectively. Therefore, early deaths due to lymphocytic lymphoma would have a tremendous negative effect on the incidence of late-developing lesions.

Hemangiosarcomas of the heart, which also accounted for some of the early deaths, were significantly increased in most stop-exposure groups compared with the controls. The highest incidence, which was about twice as high as that of other groups, occurred in the group exposed to 312 ppm, followed by the groups exposed to 200 ppm and 625 ppm (26 weeks). The lowest incidence occurred in the group exposed to 625 ppm for 13 weeks. Hemangiosarcomas appeared at about 9 months in the 200, 312, and 625 ppm (26-week) stop-exposure groups. Comparison (life table test) of groups having the same total exposures showed that the incidences of hemangiosarcomas in mice exposed to 625 ppm were significantly lower than that of the corresponding group exposed to 312 ppm (p=0.032) but not 200 ppm. The incidences of hemangiosarcomas in both 625-ppm stop-exposure groups were higher than that in the 625-ppm 2-year exposure group, probably due to longer survival of the stop-exposure groups.

The incidences of neoplastic lesions of the lung (alveolar/bronchiolar adenoma, adenocarcinoma, or carcinoma) were significantly elevated in each exposure group. The highest incidence occurred in the 200-ppm stop-exposure group, followed by the 312-, 625- (13 weeks),

and 625-ppm (26 weeks) groups. The adenomas developed after week 47 and the adenocarcinomas and carcinomas developed after week 53; the late appearance of these lesions relative to lymphocytic lymphomas probably accounted for the lowest incidence of lung neoplasms occurring in 625 ppm (26 weeks) group. A life table analysis suggested the incidence of lung lesions in the 625 ppm (26 weeks) group was significantly *greater* than in the 312 ppm (52 weeks) group (p=0.013), but no difference was detected between the 200 ppm (40 weeks) and 625 ppm (13 weeks) groups.

Mice exposed to 200 ppm 1,3-butadiene for 40 weeks had significantly increased incidences of hepatocellular adenomas and adenomas/carcinomas combined; the incidences of hepatocellular carcinomas analyzed alone were not significantly increased. Exposure to 1,3-butadiene at 312 ppm or 625 ppm (13 or 26 weeks) did not increase the incidence of hepatocellular neoplasms of any type. The earliest detection of these neoplasms was 67 weeks for the 625 ppm (13 weeks), 57 weeks for the 200 ppm, 47 weeks for the 312 ppm, and 45 weeks for the 625 ppm (26 weeks) stop-exposure groups. A logistic regression analysis found no differences between the 200 ppm and 625 ppm (13 weeks) or the 312 ppm and 625 ppm (26 weeks) groups.

A low incidence of squamous cell papillomas of the forestomach occurred in each of the groups, and squamous cell carcinomas were seen in mice exposed to 312 ppm or 625 ppm for 13 and 26 weeks. The incidences of squamous cell papillomas were not significantly greater than controls for any group, but the incidences of squamous cell carcinomas were significantly greater by the life table test, which is considered to be the appropriate test (NTP, 1993) for these fatal neoplasms. A life table analysis also revealed a statistically significant exposure-rate effect for the squamous cell carcinomas in both of the total exposure groupings (p=0.019 for 8,000 ppm·weeks and p=0.015 for 16,000 ppm·weeks), suggesting that the higher concentration/shorter duration exposures were more potent.

The incidence of adenomas of the Harderian gland was significantly greater in each exposure group than in the controls by a logistic regression test. A low incidence of Harderian gland carcinomas occurred in mice exposed to 200 ppm for 40 weeks (not significant), 312 ppm for 52 weeks (p=0.006), and 625 ppm for 13 weeks (not significant). No Harderian gland carcinomas were observed in the controls or in mice exposed to 625 ppm for 26 weeks. A logistic regression analysis did not detect any exposure-rate effects.

Other neoplasms occurred at low incidence in the stop-exposure studies; they were considered to be related to exposure because of their low spontaneous incidences in NTP historical control male mice. These neoplasms occurred in the kidney, brain, Zymbal's gland, and preputial gland. The incidences of these neoplasms are also summarized in Table 6-8.

Renal tubule neoplasms occurred in historical male control mice; the range was 0 to 1%. The small number of these neoplasms in each of the exposure groups are considered to be related

to administration of 1,3-butadiene because the incidences were greater than the upper range for historical controls.

Brain neoplasms, including two neuroblastomas and two malignant gliomas, observed in male mice exposed to 625 ppm for 13 weeks and one malignant glioma observed in male mice exposed to 625 ppm for 26 weeks may have been related to 1,3-butadiene exposure. Brain neoplasms are rare in untreated $B6C3F_1$ mice; none have been reported in 574 NTP historical control male mice. Furthermore, a low incidence of gliomas was also reported in the previous NTP (1984) study. For these reasons, the brain neoplasms are considered exposure-related lesions.

A low incidence of preputial gland carcinomas occurred in the exposed groups in the stop-exposure studies, and none were seen in controls. Compared with the incidence in concurrent controls, the combined incidences of preputial gland tumors (adenoma and carcinoma) were significant in male mice exposed to 312 ppm (52 weeks) and to 625 ppm (13 and 26 weeks) by the life table test. Preputial gland carcinomas were not reported in a survey of NTP historical control mice, further indicating that these neoplasms are probably related to exposure to 1,3-butadiene.

One male exposed to 200 ppm for 40 weeks, two males exposed to 625 ppm for 13 weeks, and two males exposed to 625 ppm for 26 weeks developed Zymbal's gland carcinomas. This lesion did not occur in male mice exposed to 312 ppm for 52 weeks; one control male, however, developed an adenoma. The combined incidence of Zymbal's gland adenomas and carcinomas in animals exposed to 625 ppm for 26 weeks was significantly increased compared with controls by the life table test. Zymbal's gland neoplasms are rare spontaneous neoplasms that had not been observed in any NTP historical controls before the only occurrence of this adenoma in the control male mice for these studies.

To summarize the results of the stop-exposure study pertaining to the relationship between exposure level and duration of exposure: For lymphocytic lymphomas, there is strong evidence that higher concentration/shorter duration exposures are more potent than the lower concentration/longer duration exposures for both the 8,000 ppm-weeks and 16,000 ppm-weeks total exposure groupings. There is also some evidence for a similar exposure-rate effect for forestomach squamous cell carcinomas in both total exposure groupings. Any exposure-rate effects at other sites are less clear, especially because it is difficult to distinguish a small apparent increased potency effect of higher concentration/shorter duration exposures from an effect of longer potential postexposure follow-up times following the shorter-duration exposures.

6.3.3. Summary of NTP (1993) Study

The 2-year inhalation study showed that 1,3-butadiene is a potent carcinogen in mice at all concentrations evaluated. It also demonstrated that exposure to lower concentrations of 1,3-butadiene than those used in the previous NTP (1984) study allowed expression of neoplasms at

other sites and provided clearer exposure-response relationships because of increased survival. Statistically significant increases in the incidences of malignant tumors at one or more sites occurred in male mice exposed to ≥20 ppm and in females exposed to ≥6.25 ppm (the lowest exposure concentration used) 1,3-butadiene for periods up to 103 weeks. The possibility, therefore, exists that lower exposure concentrations would also cause cancer in $B6C3F_1$ mice. The percentage of animals bearing malignant tumors increased from about 30% in the controls to nearly 90% in the highest exposure group, 625 ppm. Lymphocytic lymphomas, hemangiosarcomas of the heart, lung neoplasms, and neoplastic lesions of the forestomach, mammary gland, ovary, and liver, lesions identified in the NTP (1984) study, were again increased in this study. In addition, the Harderian gland and preputial gland were identified as sites of 1,3-butadiene-induced neoplasms. Tumors observed in the kidneys, skin, Zymbal's gland, and intestine may also have been related to 1,3-butadiene exposure.

The stop-exposure study demonstrated that limited exposure to 1,3-butadiene also induces neoplasms at multiple organ sites in male $B6C3F_1$ mice. Incidences of lymphocytic lymphomas, hemangiosarcomas of the heart, alveolar-bronchiolar neoplasms, forestomach squamous cell neoplasms, Harderian gland neoplasms, and preputial gland neoplasms were increased compared with controls after exposure to 625 ppm 1,3-butadiene for only 13 weeks. The stop-exposure study also demonstrated an apparent exposure-rate effect for the induction of lymphocytic lymphomas by 1,3-butadiene. At equivalent total exposures, the induction of lymphocytic lymphomas was greater with exposure to a higher concentration of 1,3-butadiene for a shorter time than for exposure to a lower concentration for a longer duration.

Overall, the NTP (1993) was a very well conducted study with a precise and comprehensive presentation of the data. Adequate numbers of animals of both sexes were exposed to multiple concentration levels of 1,3-butadiene for a major portion of their life span. Comprehensive histopathological evaluations were performed and mortality and tumor incidences were analyzed statistically using multiple methods.

6.3.4. 1-Year Study (Irons et al., 1989; Irons, 1990)

To elucidate the mechanism of murine leukemogenesis, Irons and coworkers compared the induction of thymic lymphomas and expression of murine leukemia virus in NIH Swiss male mice and $B6C3F_1$ male mice by exposing them to 1,250 ppm 1,3-butadiene, 6 h/day, 5 days/week for 52 weeks. Activation of an endogenous esotropic retrovirus has been associated with spontaneous lymphomas in the $B6C3F_1$ mouse. The NIH mouse strain was used because it does not express the esotropic murine leukemia viruses expressed in $B6C3F_1$ mice. The background rate for thymic lymphoma in NIH mice is nearly zero. Although there was a marked difference between the incidence of thymic lymphoma/leukemia in $B6C3F_1$ mice (57%) and the incidence in similarly

exposed NIH mice (14%), the study showed that 1,3-butadiene can induce thymic lymphomas independently of an activated retrovirus. In addition, because these studies were for only 52 weeks, they did not necessarily allow for a full response for induction of lymphomas by 1,3-butadiene.

6.4. RELATED COMPOUNDS

The draft report on the toxicology and carcinogenicity of 4-vinyl-1-cyclohexene, a dimer of 1,3-butadiene, was reviewed in U.S. EPA (1985). The final report (NTP, 1986) contains the same information; therefore, the data are not summarized in this update. The basic conclusion was that there was clear evidence of carcinogenicity of 4-vinyl-1-cyclohexene (by gavage) in female mice based on increased ovarian neoplasms and equivocal evidence in male mice based on marginal increases of malignant lymphomas and alveolar/bronchiolar adenomas. In rats, there was inadequate evidence in males, at least in part because of excessive mortality, and equivocal evidence in females based on increased neoplasms of the clitoral gland.

The 1,3-butadiene metabolites 1,2-epoxy-3-butene and 1,2:3,4-diepoxybutane have been shown to be carcinogenic in rats when administered by skin application or subcutaneous injection (van Duuren et al., 1963, 1966). In addition, 1,2-epoxybutane, a related compound that is used as a stabilizer for chlorinated hydrocarbon solvents, was administered by inhalation 6 h/day, 5 days/week for 24 months at exposure concentrations of 0, 200, or 400 ppm to F344/N rats and 0, 50, or 200 ppm to B6C3F$_1$ mice (Dunnick et al., 1988). The treatment and control groups consisted of 50 male and 50 female animals of each species. Exposure-related inflammatory, degenerative, and proliferative lesions occurred in the nasal cavity of both rats and mice. Neoplastic lesions were restricted to the respiratory tract in rats. At 400 ppm, nasal papillary adenomas were observed in seven male rats and in two female rats; none were observed in controls. In male rats exposed to 400 ppm, there was also an increased incidence of alveolar/bronchiolar adenomas or carcinomas (combined) (5/50) compared with controls (0/50). No exposure-related neoplastic lesions were seen in male or female mice.

6.5. DISCUSSION AND CONCLUSIONS

Previous long-term inhalation studies have shown that 1,3-butadiene is carcinogenic in rats and mice, inducing tumors at multiple organ sites (NTP, 1984; Owen et al., 1987). Results of the 2-year inhalation study (NTP, 1993) presented in this report confirmed the carcinogenicity for 1,3-butadiene in male and female B6C3F$_1$ mice as demonstrated in an earlier study (NTP, 1984).

Of particular interest in this study were the large number of primary organ sites of tumor induction by 1,3-butadiene; the early and extensive development of lymphomas; the induction of uncommon tumors, such as hemangiosarcomas of the heart and squamous cell neoplasms of the forestomach; and the development of malignant lung tumors at exposure concentrations as low as

6.25 ppm. Because there were no exposure levels of 1,3-butadiene at which a carcinogenic response was not induced, it is likely that exposure to concentrations below 6.25 ppm would also cause cancer in mice.

Exposure to 1,3-butadiene at concentrations ranging from 6.25 to 625 ppm for 2 years caused increased incidences of neoplasms in the hematopoietic system, heart, lung, forestomach, mammary gland, ovary, and liver, all lesions identified in the NTP (1984) study. The Harderian gland and preputial glands were identified as additional sites, and tumors in the kidneys, skin, Zymbal's gland and intestine were marginally associated with 1,3-butadiene. Because of increased survival, the study also established clearer concentration-response relationships than the 1984 study. Competing risks of early-developing lethal lymphocytic lymphomas at high concentrations preempted the appearance of late-developing neoplasms at some organ sites.

Separate experiments with reduced exposure durations (stop-exposure study) showed that continued exposure is not necessary for development of neoplasms. The incidences of lymphocytic lymphomas, hemangiosarcomas of the heart, and tumors of the lung, forestomach, Harderian gland, and preputial gland were increased in mice exposed for only 13 weeks to 625 ppm 1,3-butadiene and it is likely that even shorter exposure durations would have produced a carcinogenic response. The stop-exposure study also showed that the concentration is a greater contributing factor in the development of lymphocytic lymphomas than the duration of exposure. At comparable total exposures, the incidence of lymphocytic lymphomas was greater with exposure to a high concentration of 1,3-butadiene for a short time compared with a lower concentration for a longer duration.

A morphological continuum of 1,3-butadiene-induced proliferative lesions to neoplasia or the progression of benign to malignant neoplasms was evident for a number of sites in both the 2-year and the stop-exposure study (NTP, 1993). Increased incidences of proliferative, nonneoplastic lesions (hyperplasia) of the cardiac endothelium, alveolar epithelium, forestomach epithelium, germinal epithelium and granulosa cells of the ovaries, mammary gland, and Harderian gland probably represent treatment-related preneoplastic changes at these target sites. The distinction between adenoma and carcinoma further reveal the biological progression of the benign lesions to malignant neoplasia. For example, in the lungs of male mice, progression from alveolar-bronchiolar adenoma to carcinoma was evident in the 200-ppm exposure group and in all of the stop-exposure groups.

The mechanism of 1,3-butadiene-induced carcinogenicity is not known; however, metabolism likely involving two reactive metabolites, 1,2-epoxy-3-butene and 1,2:3,4-diepoxybutane, is thought to be an important factor (Chapters 3 and 4).

Results of previous carcinogenicity studies reviewed in U.S. EPA (1985) have shown different effects of exposure to 1,3-butadiene in rats and mice, with mice being more sensitive to

the induction of carcinogenic effects than rats. The carcinogenic activity in Sprague-Dawley rats exposed to 1000 or 8000 ppm 1,3-butadiene was largely limited to endocrine tissues or hormonal responsive tissues, such as pancreas, Leydig cells of the testis, uterus, Zymbal gland, mammary gland, and thyroid (Owen et al., 1987), whereas exposure of $B6C3F_1$ mice to much lower concentrations of 1,3-butadiene caused significantly increased incidences of mammary gland neoplasms and granuloma cell neoplasms of the ovary as well as malignant lymphomas, hemangiosarcomas of the heart, alveolar-bronchiolar neoplasms, squamous cell neoplasms of the forestomach, and hepatocellular neoplasms. The reason for the species difference is not known, but may in part be due to differences in toxicokinetics.

Toxicokinetic studies have shown species-related quantitative and qualitative differences in the metabolism and disposition of inhaled 1,3-butadiene that may, in part, account for the observed species variability in the toxicity (Chapter 3). For example, metabolism studies have shown that blood concentrations of 1,3-butadiene are higher in mice than in rats, and are lower in monkeys than in either rodent species. In vitro studies using liver microsomes have shown that the metabolism of the reactive intermediate, 1,2-epoxy-3-butene, to the non-DNA-reactive 1,2-dihydroxybut-3-ene is the prevalent pathway in human and rat preparations, whereas mouse liver microsomes convert 1,2-epoxy-3-butene to DNA-reactive 1,2:3,4-diepoxybutane in addition to the nonreactive 1,2-dihydroxybut-3-ene (Csanáday and Bond, 1991).

Investigations by Irons and coworkers (Irons et al., 1989; Irons, 1990) to explain the species differences of 1,3-butadiene-induced carcinogenicity have focused on the possibility that activation of an endogenous leukemia retrovirus may play a critical role in 1,3-butadiene-induced lymphoma in $B6C3F_1$ mice. The incidence of thymic lymphomas was greater in $B6C3F_1$ mice (57%) than in NIH Swiss mice (14%) exposed to 1,250 ppm 1,3-butadiene for 1 year. However, the NIH Swiss mouse does not express the endogenous leukemia retrovirus and has a very low background rate for thymic lymphomas. Thus, the finding that exposure to 1,3-butadiene caused a 14% incidence of thymic lymphomas in NIH Swiss mice suggests that 1,3-butadiene can induce thymic lymphomas independently of an activated retrovirus.

Identification of activated oncogenes in chemically induced tumors also may provide information regarding the mechanism of tumor induction by butadiene. For example, because K-ras is the most commonly detected oncogene in human cancers, tumors from the NTP (1993) study were evaluated for the presence of K-ras oncogenes (Goodrow et al., 1990). Activated K-ras oncogenes were detected in 6/9 lung tumors, 3/12 hepatocellular carcinomas, and in 2/11 lymphomas obtained from $B6C3F_1$ mice exposed to 1,3-butadiene at concentrations ranging from 62.5 to 625 ppm. A specific codon 13 mutation was found in most of the activated K-ras oncogenes, suggesting a chemical-specific effect. Activated K-ras genes have not been found in spontaneously occurring liver tumors or lymphomas (Goodrow et al., 1990) and were observed only in 1/10 of spontaneous

lung tumors in $B6C3F_1$ mice (Goodrow et al., 1990; Reynolds et al., 1987). Furthermore, it was shown that tumor suppressor genes are inactivated during 1,3-butadiene carcinogenesis. Soderkvist et al. (1992) identified allelic losses in the p53 tumor suppressor gene in lung and mammary carcinomas and lymphomas of $B6C3F_1$ mice exposed to 1,3-butadiene, that were analogous to those observed in a variety of human cancers.

Immune-function assays conducted by Thurmond et al. (1986) in which $B6C3F_1$ mice were exposed by inhalation to 1,250 ppm 1,3-butadiene for 6 or 12 weeks showed that 1,3-butadiene exerts no significant immunosuppressive effects, suggesting that 1,3-butadiene causes neoplasia by mechanisms other than by compromise of immune function.

In addition to the carcinogenic effects noted in the NTP (1993) study, exposure to 1,3-butadiene caused hematological changes indicative of a partially regenerative anemia in mice exposed to $\geq$62.5 ppm 1,3-butadiene. Mice exposed to 625 ppm exhibited bone marrow atrophy and splenic and hepatic extramedullary hematopoiesis. Increases in mean cell volume and mean cell hemoglobin at 625 ppm 1,3-butadiene suggested that although 1,3-butadiene caused suppression of hematopoiesis in the bone marrow, younger larger cells may have been released into the blood from extramedullary sites. A macrocytic-megaloblastic anemia was reported in $B6C3F_1$ mice exposed to 1,250 ppm 1,3-butadiene for 6 weeks (Irons et al., 1986a, b).